USE OF BLUE-GREEN ALGAE

TO IMPROVE THE CHEMICAL QUALITY OF MUNICPAL SOLID WASTE COMPOST

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Executive Summary

Compost as a soil amendment is widely used in agriculture, improving soil by adding more nutrients and organic matter and by adjusting the carbon to nitrogen ratio of the soil. Because of the harsh environment and barren soil conditions in Newfoundland and Labrador, the generation and application of high-quality composting products are receiving more attention. Conventional composting processes have been well developed, as they have been studied for many years. However, the use of blue-green algae in composting by mixing multiple waste sources has only recently been studied. The three waste sources utilized in this research are sludge from the municipal wastewater treatment system in St. John's, fly ash from power plants, fish waste from local fish-processing industries; and the algae Anabaena strain 387.

This research investigated the potential application of blue-green algae in improving the quality of compost generated from multiple waste streams, including fly ash, fish waste, and sludge from a local wastewater treatment plant. Algae strain Anabaena 387 was obtained from the Canadian Phycological Culture. Various ratios of fly ash, fish-waste compost, sludge, and algae were tested at different reaction periods to generate different levels of compost products. The parameters, including trace elements, carbon to nitrogen ratio, pH, moisture, organic matter, and germination index, were monitored to evaluate the compost quality. Three levels of compost products were generated: Type AA, A, and B. Algae-treated compost can be used as an amendment for agriculture applications.

This research provides an innovative method for the generation of different levels of compost products. The contributions of this research include:

1) Compared with the conventional composting process, multiple resources were used: fly ash, sludge, and barren fish-waste compost after extraction;
2) The reuse of these multiple resources offers another environmentally friendly way for waste management and recycling;
3) This research shows how an environmental problem (algae bloom) can be used as a biotechnology (biodegradation) to generate a valuable agricultural product (compost); and
4) Multiple choices of compost products were available. Type B level or Type A level compost can be used for horticultural purposes, and Type AA level compost can be applied in agriculture. Different demands from customers can be met by a diverse selection.

Although this research brings a new scope to the study of compost, considerable work is needed to reveal a deeper understanding of biodegradation processes. Here are some recommendations for future research.

For the chemical qualities of the compost, this research mainly focused on the improvement of the carbon to nitrogen ratio, organic matter, and nitrogen content. However, trace elements also affect the classification of compost. Due to difficulties in separating algae from the compost, the concentration of trace elements in the compost did not decrease after biodegradation. It is recommended that more work be designed in order to reduce the concentration of trace elements in the samples.

In addition, the biodegradation process can be set up as a modeling program for a better control of reaction factors. Flexible adjustments of reaction conditions during the process might help with generating better compost products with less energy input and a shorter production period.

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CHAPTER 1 INTRODUCTION

1.1 Background

Compost as a soil amendment is widely used in agriculture, improving the soil by adding more nutrients and organic matter and by adjusting its carbon to nitrogen ratio. Because of the harsh environment and barren soil conditions in Newfoundland and Labrador, the generation and application of high-quality composting products are receiving more attention. Conventional composting processes have been well developed, as they have been studied for many years. However, the use of blue-green algae in composting by mixing multiple waste sources has only recently been studied. The three waste sources utilized in this research are sludge from the municipal wastewater treatment system in St. John's, fly ash from power plants, fish waste from local fish-processing industries, and the algae Anabaena strain 387 found in abundance in ponds in the Avalon Peninsula.

Sludge from the wastewater treatment plant has always been a serious environmental concern in the City of St. John's. Usually after the dewatering process, the remaining solids are compressed and disposed directly into the landfill. According to the City's official website, around 65 tons of solid waste are produced and end up as landfill disposal every year. Many complaints have come from a nearby community about the odour of this dewatered sludge. In addition, these wastes occupy a large area in the landfill. Basically, this dried sludge is bio-solids or organic materials, which can be further biodegraded and used as a compost amendment for agricultural purposes.

One innovative approach to converting sludge into compost is to treat it with blue-green algae, also known as cyanobacteria. These bacteria caused blooms in several Northeast Avalon ponds in Newfoundland (Government of Newfoundland and Labrador, 2007). These photosynthetic bacteria have a high carbon content in the form of soluble sugars, the most common glycogen (Table 1), which can occupy half of the cellular area; this quantity is greater than the amount of sugar in rice, 14.1% (Table 1). The carbon in blue-green algae is highly stable; it is a water-soluble polysaccharide, it is non-radioactive, and it can break down enzymatically into simpler carbon compounds to produce cellular energy.

Table 1 Distribution of Sugars in BGA Strains (Nakamura et al., 2005; Wolk, 1973)

Blue-green algae species	**Strain number**	**Type of polysaccharide**	**% area distribution**
Anabaena sp.	PCC7120	Glycogen	49.8
Dermocarpa sp.	MBIC10001	Glycogen	39.7
Fischerella major	NIES-N-592	Glycogen	34.8
Oscillatoria limnetica	NIES-N-36	Glycogen	47.5
Phormidium sp.	MBIC10025	Glycogen	56.3
Spirulina platensis	IAM-M-135	Glycogen	42.6
Synechococcus sp.	PCC7942	Glycogen	61.7
Anabaena cylindrica	---	Mannose	50
Myxosarcina burmensis	IAM-M-246	Semi-amylopectin	26.7
Cyanobacterium sp.	MBIC10216	Semi-amylopectin	19.4
Rice (*Oryza sativa*, L.)	---	Amylopectin	14.1

Blue-green algae have structures called heterocysts that allow them to fix nitrogen (Figure 1). They convert atmospheric nitrogen into amino acids, which are utilized by their cells, and they can remove heavy metals from the environment by chemisorption (Bender et al., 1994; Chojnacka et al., 2005; DePhillipis et al., 2011). Blue-green algae are also capable of polycyclic aromatic hydrocarbon (PAHs) bioremediation (Sorkhoh et al., 2005; Al-Mailem et al., 2010). They also contain lipids, the most common being glycolipids (Kenyon, 1972), which can emulsify these water-insoluble pollutants for bioremediation.

Fly ash, a worldwide environmental problem, is the main by-product generated by oil- and coal-burning power plants (Ahmaruzzaman, 2009; Scheetz and Earle, 1998; Zacco et al., 2014). Fly ash consists of fine particles ranging in size from 10 μm to 100 μm and, because of its low density (0.25-0.4 g/cm^3), the improper management of fly ash leads to air pollution and human respiratory disease (Scheetz and Earle, 1998; Wang and Wu, 2006). An important characteristic of fly ash that can be used advantageously is its high carbon content (50-90% by weight). If fly ash can be utilized as an amendment to compost products, it not only provides an environmentally friendly method for recycling fly ash but it also generates a useful soil ameliorant.

Fish waste has been considered the perfect composting material (Lopez-Mosquera et al., 2011). The fishery in Newfoundland has had a long history and today plays an important role in economic development in Newfoundland and Labrador. Fish waste generated from seafood manufacturing firms is usually rich in nutrients, especially nitrogen and phosphorus, and this waste can decompose quickly. These properties make fish waste a good agricultural fertilizer.

The *algae* used in this research is Anabaena, strain 387. Blue-green algal biodegradation makes Anabaena an ideal candidate for improving the chemical qualities of compost. Since blue-green algae also causes blooms, including those in several Northeast Avalon ponds, this research is an example of how natural agents can produce biotechnological products, in this case how a blue-green algae bloom, an environmental agent, can be used to improve the qualities of compost for agricultural use.

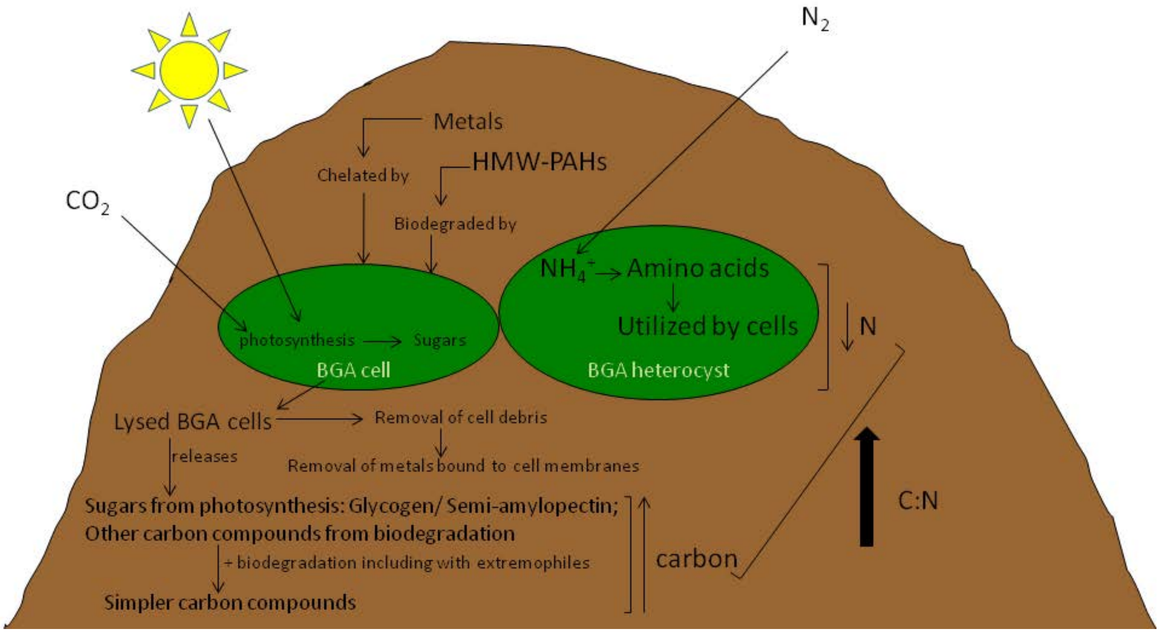

Figure 1.1 Theoretical Approach to Improving the Quality of MSW Compost using BGA

1.2 Objectives

This research attempts to provide an innovative method for generating a qualified compost product with different levels of applications and also to determine how compost quality could be affected by algal biodegradation. Meanwhile, the quantifications of six evaluation parameters (i.e., trace elements, carbon to nitrogen ratio, pH, moisture, organic matter, and germination index) are used as evidence for explaining and illustrating the relationship between compost and algae. These objectives entail the following major research tasks:

1) Collect raw materials (sludge, fly ash, and fish waste) from different resources and learn how to culture Anabaena in the laboratory;
2) Characterize each raw material in order to have a good understanding of each from quantitative and qualitative points of view;
3) Mix raw materials in different ratios and characterize each sample to determine a reasonable mixing ratio;
4) Biodegrade the compost samples by adding different doses of algae solution in order to find the optimal amount; and
5) Draw a biodegradation curve of the process in order to ascertain the optimal reaction time.

The results from this research will clarify the function of Anabaena and the interaction between algae and compost. This research will contribute to the existing need for waste management in Newfoundland and Labrador and help with the setting up a compost program at the Robin Hood Bay Facility in the near future. It will also decrease the possible health risks associated with airborne pollutants.

1.3 Rationale

This research contributes to the objectives of the waste management with the following rationale:

1. *To stimulate research in solid waste management issues and to incorporate community needs into research projects*

Newfoundland and Labrador's waste management strategies in 2002 (Government of Newfoundland and Labrador, 2002) include waste diversion and modernizing standards and technology for waste management including those for composting. This research on the use of blue-green algae to improve the chemical qualities of municipal solid waste (MSW) compost has contributed to waste management strategies. This is also an innovative approach to improving compost as a fertilizer amendment to increase biofuel production.

2. *Provide research evidence to inform policy making and to assist community groups in their local decision-making*

This research project provides research methods, data, and evidence for how blue-green algae can be used to improve the chemical composition of MSW compost. This information can be useful for similar biotechnological research at Memorial University of Newfoundland, the Newfoundland and Labrador Waste Management Committee, the Robin Hood Bay Facility, and other waste management facilities as a model for waste management. Since blue-green algae also cause blooms, including the bloom in 2007 in several Northeast Avalon ponds (Government of Newfoundland and Labrador, 2007), this research project will be an example of how natural agents can be used to produce biotechnological products, in this case how a blue-green algae bloom, an environmental agent, can be used to improve MSW compost (another environmental agent) for agricultural use.

3. To build a network of interested partners representing a diversity of sectors

This multi-disciplinary research work will build a diverse network of academic, government, and entrepreneur partners. Specifically, this include Memorial University of Newfoundland, where this work will be done, and also the Newfoundland and Labrador Waste Management Committee, Newfoundland and Labrador Department of Environment and Conservation, and the Newfoundland Environment Industry Association for whom the results from this research work will be useful as an innovative, exemplary approach for MSW management and with whom future collaboration can be established for advanced work on this project that can help community needs in Newfoundland and Labrador, Canada.

4. To make a different in the lives of Newfoundland and Labrador people

As will be shown from this research project, the MSW compost that will be chemically improved using blue-green algae can be used as an agricultural amendment to increase the biofuel productivity from switchgrass and corn. This will help the Waste Management Facility to manage and make use of the high quantities of MSW compost. This way, the MSW compost at the Robin Hood Bay Facility will be better managed and lead to decreased possible health risk from airborne pollutants and prolonged compost stench.

1.4 Organization

Chapter 2 presents the experimental design and standard methods for the six evaluation parameters—trace elements, carbon to nitrogen ratio, pH, moisture, organic matter, and germination index—used in this research. Chapter 3 includes the important data and detailed discussions about the biodegradation curve and optimal dose of algae. Conclusions and recommendations for future work are presented in the last chapter.

CHAPTER 2 RESEARCH METHODOLOGY

2.1 Algal Culturing

The blue-green algae used in this research, Anabaena strain 387, was the likely cause of the blue-green algae bloom in Newfoundland in 2007 (Government of Newfoundland and Labrador, 2007). Anabaena strain 387 can be obtained from the Canadian Phycological Culture Centre (CPCC) from their list of cultures (CPCC, 2011) and cultured in the laboratory.

Modified Bold’s Basal Medium (3NBBM) was selected as the optimal medium for culturing Anabaena; the recipe for 3NBBM is given in Table 2.1. Figure 2.1 shows the algal solution using 3NBBM.

Figure 2.1 Anabaena Strain 387 in Modified Bold’s Basal Medium Solution

Table 2.1 Recipe for Modified Bold's Basal Medium (3NBBM)

Modified Bold's Basal Medium (3NBBM) Autoclave. The final pH should be 6.6. Final volume is 1000 mL.				
Item	**Component**	**Stock Solution (g/L dH_2O)**	**Quantity Used**	**Concentration in Final Medium (M)**
1	$NaNO_3$	75.00	**10 mL**	$8.82*10^{-3}$
2	$CaCI_2$-$2H_2O$	2.50		$1.70*10^{-4}$
3	$MgSO_4$-$7H_2O$	7.50		$3.04*10^{-4}$
4	K_2HPO_4	7.50		$4.31*10^{-4}$
5	KH_2PO_4	17.50		$1.29*10^{-3}$
6	NaCl	2.50		$4.28*10^{-4}$
	Alkaline EDTA Solution			
7	EDTA	5.00		$1.71*10^{-4}$
8	KOH	3.10		$5.53*10^{-4}$
	Acidified Iron Solution			
9	$FeSO_4$-$7H_2O$	0.498		$1.79*10^{-5}$
10	H_2SO_4		0.1mL=100uL	
	Trace Metal Solution			
11	H_3BO_3	11.42	**1 mL**	$1.85*10^{-4}$
12	$ZnSO_4$-$7H_2O$	8.82		$3.07*10^{-5}$
13	$MnCI_2$-$4H_2O$	1.44		$7.28*10^{-6}$
14	MoO_3	0.71		$4.93*10^{-6}$
15	$CuSO_4$-$5H_2O$	1.57		$6.29*10^{-6}$
16	$Co(NO_3)_2$-$6H_2O$	0.49		$1.68*10^{-6}$

2.2 Raw Materials

Fly ash

Fly ash was obtained from an oil-fired power plant. This waste is abundant, with millions of tons generated annually from the burning of heavy fuel oil (HFO). Only a small portion of oil fly ash (OFA) is reused productively; most is dumped into landfills (Shackelford, 2000). As reported in the literature, about 3 kilograms of ash residue is generated by burning 1000 litres of HFO (Tsai and Tsai, 1997); approximately 90% of this ash passes through the flue gas stream, which is collected by air pollution control devices such as electrostatic precipitators (ESP) or cyclones (Hsieh and Tsai, 2003). On average, 50-60 tons of OFA is generated daily from a 2300 MW HFO-operated power plant (Wayne and Turner, 2009; Hsieh and Tsai, 2003).

Fish-waste compost

Fish-waste compost is a very nutritious fertilizer; however, the fish-waste compost used in this experiment can be treated as barren waste, the by-product after extraction for other purposes.

Sludge

The sludge, obtained from the Riverhead Wastewater Treatment Facility (RHWTF), is a liquid-solid mixture after the physical sedimentation and biological digestion process.

2.3 Experimental Design

2.3.1 Characterization of Raw Materials

For a better understanding of the sources used in the experiment, the characterization of the raw materials—fly ash, sludge, and fish waste—was based on the following six parameters: carbon to nitrogen ratio (C:N), germination index (GI), moisture, organic matter (OM), pH, and trace elements. The methodology for each parameter followed standard methods, described later in this chapter.

2.3.2 Different Mixing Ratios

Based on the characterization of each raw material, eight mixture ratios—R1, R2, R3, R4, R5, R6, R7, and R8—were established. After mixing, all parameters (C:N, germination index, moisture, organic matter, pH, and trace elements) were tested again. According to the classification criteria provided by the Bureau de normalisation du Québec (BNQ) as reported in (BNQ, 2005), some of these eight ratios qualify as Type B compost; others do not. Algae has the potential to biodegrade contaminants in the compost. Therefore, those samples which do not qualify might have the potential to be Type A or Type AA compost after algal treatment.

2.3.3 Biodegradation Curve

Those ratios which did not qualify were mixed with the algal solution and experienced a biodegradation process. Biodegradation behaviour was the main focus in this stage. Day 1 (D1) represents the first day that the algal solution and compost samples were mixed. As the biodegradation proceeded, six parameters were tested every six days until some parameters, such as C:N and organic matter, reached stable values. At the end of the biodegradation process, the optimal number of reaction days was clarified. Meanwhile, in order to verify the optimal dose of algae solution obtained from the last stage, three doses—5 ml, 10 ml, and 20 ml—with 20 g samples were conducted at the same time. Six runs were established, and their representations are listed below:

Run 1: 20 g R7 mixture samples with 5 ml algal solution
Run 2: 20 g R7 mixture samples with 10 ml algal solution
Run 3: 20 g R7 mixture samples with 20 ml algal solution
Run 4: 20 g R8 mixture samples with 5 ml algal solution
Run 5: 20 g R8 mixture samples with 10 ml algal solution
Run 6: 20 g R8 mixture samples with 20 ml algal solution

2.3.4 Determination of Dose of Algae

Improve the chemical characteristics of those ratios that did not qualify by using Anabaena. C:N and organic matter were the focus in this stage. In order to determine the optimal dose of Anabaena solution to be added to the mixtures, three doses (5 ml, 10 ml, and 20 ml) with 20 g mixture samples were tested for the same six parameters.

2.3.5 Classification of Composts

After the biodegradation process, different quality compost products were generated. According to the classification criteria provided by Bureau de normalisation du Quebec (BNQ, 2005) and the Canadian Council of Ministers of the Environment (CCME, 1996), qualified compost products were classified into Type AA, Type A, and Type B, respectively, while some samples probably still did not qualify for either of these types.

2.4 Sample Analysis

2.4.1 Determination of Germination Index

Phytotoxicity was determined on the basis of the germination index (GI) by using cucumber seeds on the compost extract (Mohammad et al., 2013; Tiquia and Tam, 1998). Five grams (dry weight) of compost sample was weighed and mixed with 50 ml of distilled water in a tube with a screw cap, then placed in an electric shaker (SHKA 4000, Model 4320) at 220 rpm for 30 minutes. After shaking, the compost extract was centrifuged (Sigma 2-16) at 5000 rpm for 15 min. The supernatant was then filtered through filter paper (Fisherbrand Q5, Dia 11.0 cm). The extract was sprayed over a Petri dish with one layer of filter paper. Ten cucumber seeds were planted per plate and allowed to germinate. All plates were incubated at 20°C on a tray placed in an Isotemp Waterbath. Seedling germination was recorded after five days of incubation. Control treatment was maintained using the same amount of distilled water on the filter paper inside the Petri dish. The number of germinated seeds and root growth were recorded in the same way. All compost extracts and controls were tested with three parallel samples. The mean values of relative seed germination and relative root growth were used in the calculation. The relative seed germination, relative root growth, and GI were calculated as follows:

$$\text{Relative seed germination (\%)} = \frac{\text{NO. of seeds germinated in extract}}{\text{NO. of seeds germinated in control}} \times 100$$

$$\text{Relative root growth (\%)} = \frac{\text{Mean root length in extract}}{\text{Mean root length in contrl}} \times 100$$

$$\text{GI (\%)} = \frac{(\text{\% Seed germination}) \times (\text{\% Root growth})}{100}$$

2.4.2 Determination of Carbon to Nitrogen Ratio (C:N)

A Perkin Elmer 2400 Series II CHN analyzer was used to determine the nitrogen and total organic carbon in the samples. The samples were dried for about 24 hours at 80 °C, then exposed to an acidic environment for another 24 hours to remove the inorganic carbon, and then dried for an additional 24 hours. A subsample was then weighed for 2$\pm$ 0.1 mg and loaded into a tin capsule before being placed in the CHN analyzer. Carbon and nitrogen values were given separately, and the C:N calculated by the following formula:

$$\text{Carbon to nitrogen ratio (\%)} = \frac{\text{Total organic carbon}}{\text{Total organic nitrogen}} \times 100$$

For each sample, the C:N was tested three times in parallel. The mean value of the three results was used for the data analysis.

2.4.3 Determination of pH Level

A glass electrode microprocessor pH meter (Mettler Toledo AG, 8603 Schwerzebach) was used to measure the pH value of the sample extract. One gram (dry weight) of compost sample was weighed and mixed with 10 ml of distilled water in a tube with a screw cap and placed in an electric shaker (SHKA 4000, Model 4320) at 220 rpm for 30 min. After shaking, the compost extract was centrifuged (Sigma 2-16) at 5000 rpm for 15 min. The supernatant was filtered through filter paper (Fisherbrand Q5, Dia 11.0 cm) before its pH was measured. For each sample, the pH was tested three times in parallel. The mean value of the three results was used for the data analysis.

2.4.4 Determination of Organic Matter

The organic matter in the compost can be estimated by calculating the content of volatile solids (Wichuk and McCartney, 2010). In order to clean all the organic matter in the crucible before testing, it should be placed in a muffle furnace (FD 1535M) at 550 ^{o}C for one hour. Cool down the crucible in a desiccator to room temperature and then weigh it to obtain the tare weight, recorded as W_0. A 2 to 3 g compost sample was weighed in the pre-weighed crucible, recorded as W_1, and placed in a drying oven at 103-105 ^{o}C for 24 hours. Cool down the crucible in a desiccator to room temperature and weigh it, recorded as W_2. Then the dried samples with the crucible were placed in a muffle furnace at 550 ^{o}C for 30 min. The crucible was weighed again after it was cooled down to room temperature, recorded as W_3. The organic matter content (%) is calculated as follows:

$$\text{Organic matter content (\%)} = \frac{\mathbf{W2 - W3}}{\mathbf{W1 - W0}} \times 100$$

Where: W0 = initial tare weight of crucible, in mg
W1 = weight of compost samples + crucible before drying, in mg
W2 = weight of compost samples + crucible after drying, in mg
W3 = weight of compost samples + crucible after ignition, in mg

For each sample, the organic matter content was tested three times in parallel. The mean value of the three results was used for the data analysis.

2.4.5 Determination of Trace Element Concentrations

Weigh about 100 mg of the sample and put it in a clean dry jar with a Teflon screw cap and record the weight. Add 2 ml of 8N HNO_3 and swirl, followed by adding 1 ml hydrofluoric acid (HF) and swirl again. Heat the sample on a hot plate (around 70℃) for two days. Cool the Teflon jar to room temperature and then open the cap and add 3 ml of Aqua Regia (3:1 conc. HCl : HNO_3). Replace the cap and heat on a hot plate again for two days. After it cools down to room temperature, add 2 ml of 8N HNO_3 and heat on a hot plate for 4-5 hours. Remove the jar from the hot plate and wait for it to cool down, then add 1 ml H_2O_2. Leave it on the hot plate until the bubbling stops. Then remove it from the hot plate and cool down; add another 1 ml H_2O_2 and heat for another 4-5 hours. Then remove the jar from the hot plate and transfer its contents into a clean, dry, labeled 45 ml snap seal container. Wash the Teflon jar with additional Nanopure water in the snap seal. Add Nanopure water until the solution in the snap seal weighs 45 g, and record the weight. Filter the solution using a funnel and a No.1 Whatman filter paper into another clean, dry, labeled 45 ml snap seal container. Add 0.5 g of the filtered solution to a 11 ml clean test tube and record the weight. Then analyze the solution using an Elan DRC II ICP-MS. For each sample, the concentration of trace elements was tested three times in parallel. The mean value of three results was used for the data analysis.

2.4.6 Determination of Moisture Content

Ignite a crucible in a muffle furnace (FD 1535M) at 550 °C for one hour; cool down in a desiccator to room temperature. Then weigh to obtain the tare weight, recorded as W_0. A compost sample around 2 to 3 g was weighed in the pre-weighed crucible, recorded as W_1; and then placed in a drying oven at 103-105 ℃ for 24 hours. Cool down the crucible in a desiccator to room temperature and weigh, recorded as W_2. The moisture content (%) is calculated as follows:

$$\text{Moisture content (\%)} = \frac{\mathbf{W1 - W2}}{\mathbf{W1 - W0}} \times 100$$

Where: W_0 = initial tare weight of crucible, in mg

W_1 = weight of compost samples + crucible before drying, in mg

W_2 = weight of compost samples + crucible after drying, in mg

For each sample, the moisture content was tested three times in parallel. The mean value of the three results was used for the data analysis.

CHAPTER 3 RESULTS AND DISCUSSION

3.1 Characterization of Raw Materials

The characterization of the raw materials—fly ash, sludge, and fish waste—focused on the following six parameters: C:N, germination index, moisture, organic matter, pH, and trace elements in the compost, following the standard methods described in chapter 2. The data are listed in Tables 3.1 and 3.2.

Compared with the standard requirements for fish-waste compost, the GI is not as high as 90% and contains too much moisture. The organic matter does not qualify the compost either even as Type B. The concentrations of all trace elements, however, are below the limited values. The sludge sample may have too many contaminants, as the GI is only 24.73%. Moisture and organic matter are also barriers that restrict the sludge from qualifying as compost. Meanwhile, the concentration of Cu is 110.6 ppm, which is higher than the acceptable maximum concentration of 100 ppm; and Se is 10.097 ppm, which is higher than the upper limit of 2 ppm. The carbon to nitrogen ratio (C:N) is good for both sludge and fish-waste compost. For fly ash, however, the C:N is 126.54, which is higher than the standard—less than 25. The GI is only 10.11%, which is much lower than the required value. The organic matter content is high enough for it to be classified as Type AA. The concentrations of Mo and Ni are 14.698 ppm and 876.698 ppm, both of which exceed the limited concentrations of 5 ppm and 62 ppm, respectively. In summary, each raw material does not qualify as compost when used by itself. However, each raw material has its own special characteristics and potentials that generate a good-quality compost product after it is mixed with the other raw materials in a certain ratio.

Table 3.1 Raw Materials Index

Raw Materials	C:N	GI (%)	Moisture (%)	Organic Matter (%)	pH	Trace Elements
Standards	**< 25**	**> 90**	**< 60**	**Type AA > 50; Type A > 40; Type B > 30;**	**NA**	See Table 3.2
Fish-Waste Compost	12.03	61.62	66.73	26.87	8.93	
Sludge	13.32	24.73	92.45	5.19	5.15	
Fly Ash	126.54	10.11	0.15	96.26	3.1	

Table 3.2 Trace Elements in Raw Materials and Standard Values (ppm)

Trace Elements	Types AA and A	Type B	Fly Ash	Sludge	Fish-Waste Compost
As	13	75	0.728	1.888	6.988
Co	34	150	0.815	1.79	0.831
Cr	210	1060	7.835	17.369	3.672
Cu	100	757	< LD*	110.6	7.95
Mo	5	20	14.698	3.119	0.307
Ni	62	180	876.698	8.87	3.436
Se	2	14	< LD*	10.097	< LD*
Zn	500	1850	13.74	162.83	78.855
Cd	3	20	< LD*	< LD*	0.698
Hg	0.8	5	< LD*	0.018	< LD*
Pb	150	500	2.531	19.295	3.864

Note: < LD* means the concentration is lower than the detection limit.

3.2 Determination of Mixing Ratios

Based on the characteristics of each raw material, eight ratios were established. Table 3.3 shows the corresponding mixture ratio in percentage, and 20 g was set as the final sample mass.

As shown in Figure 3.1, fish-waste compost accounts for the major percentage and fly ash the lowest for each individual sample. The high concentration of organic matter (OM) in fly ash can contribute to improving the overall OM content in the mixtures; meanwhile, the high C:N and over-the-limit concentrations of heavy metals should be considered. The high moisture content in sludge also needs to be considered when adjusting the eight ratios. R6 has the highest percentage for both sludge and fly ash, which is supposed to generate the lowest quality compost; however, R7 has the highest percentage of fish-waste compost and relatively lower sludge and fly ash content, which is supposed to generate the best compost. After mixing fly ash, sludge, and fish-waste compost in eight different ratios, the characterizations for each ratio were conducted in the same way. The indexes of the eight ratios are listed in Tables 3.4 to 3.11.

Table 3.3 Mixture Ratios (% of 20 g mixture)

Mixture	Fish-waste compost (%)	Slurry (%)	Fly ash (%)
R1	88	10	2
R2	80	10	10
R3	70	20	10
R4	80	14	6
R5	76	16	8
R6	50	40	10
R7	90	6	4
R8	79	14	7

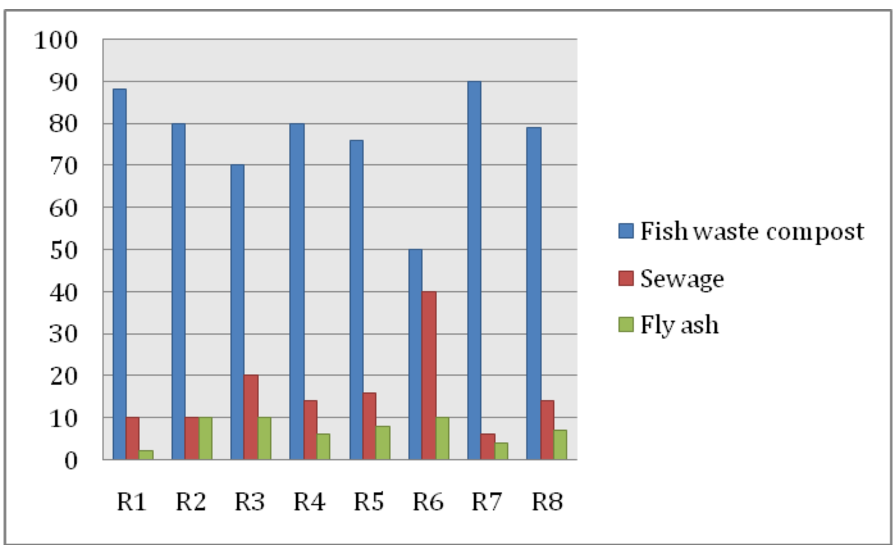

Figure 3.1 Mixture Ratios for Eight Samples

For R1, the GI, C:N, and moisture content meet the standard, especially with its high OM content. However, the concentration of Se is 8.846 ppm, which is higher than 2 ppm (Type AA and A level) but lower than 14 ppm (Type B level). R1, then, can be classified as a Type B level compost, even though the OM is higher than 50% (Type AA level). For R2, the GI, C:N, and moisture content qualify it for compost application. However, the concentration of Ni is 107.764 ppm, which is higher than 62 ppm (Type AA and A level) but lower than 180 ppm (Type B level). The concentration of Se is 4.45 ppm, which is higher than 2 ppm (Type AA and A level) but lower than 14 ppm (Type B level). Therefore, R2 can also be classified as a Type B level compost, even though the OM is 60.36% (Type AA level). A similar situation exists for R4; the concentration of Se is 2.004 ppm, which is a little higher than 2 ppm (Type AA and A level) and lower than 14 ppm (Type B level). So it can still be classified as a Type B level compost. All other parameters qualify.

Unfortunately, R3, R5, and R6 do not qualify as compost products because of their high C:N and high concentrations of trace elements. According to CCME (1996) and BNQ (2005) standards, the C:N should be lower than 25. For R3, R5, and R6, the C:N is 28.14, 42.65, and 35.27, respectively. Based on the restrictions for trace elements, the maximum acceptable concentration of Ni is 180 ppm; in R3, R5, and R6, however, the Ni content is 182.507 ppm, 237.831 ppm, and 302.525 ppm, respectively. For R7 and R8, all parameters meet the requirements. According to the standard values for the concentration of trace elements and the OM content, both R7 and R8 can be classified as a Type A level compost.

Table 3.4 Characterizations for Ratio 1 (R1)

	Standards			R1
Organic carbon	NA			37.64
Nitrogen	NA			2.82
C:N	< 25			13.35
GI (%)	> 90			158.08
Moisture (%)	< 60			32.46
Organic Matter (%)	Type AA > 50	Type A > 40	Type B > 30	61.40
pH	NA			8.14
Trace Elements	Type AA	Type A	Type B	R1
As	13		75	7.547
Co	34		150	0.844
Cr	210		1060	6.580
Cu	100		757	20.718
Mo	5		20	1.525
Ni	62		180	28.220
Se	2		14	8.846
Zn	500		1850	92.525
Cd	3		20	0.561
Hg	0.8		5	< LD*
Pb	150		500	4.768

Table 3.5 Characterizations for Ratio 2 (R2)

	Standards			R2
Organic carbon	NA			52.98
Nitrogen	NA			2.77
C:N	< 25			19.12
GI (%)	> 90			153.11
Moisture (%)	< 60			31.67
Organic Matter (%)	Type AA > 50	Type A > 40	Type B > 30	60.36
pH	NA			8.17
Trace Elements	Type AA	Type A	Type B	R2
As	13		75	6.080
Co	34		150	0.576
Cr	210		1060	6.810
Cu	100		757	15.280
Mo	5		20	3.374
Ni	62		180	107.764
Se	2		14	4.450
Zn	500		1850	71.210
Cd	3		20	0.399
Hg	0.8		5	< LD*
Pb	150		500	3.371

Table 3.6 Characterizations for Ratio 3 (R3)

	Standards			R3
Organic carbon	NA			51.21
Nitrogen	NA			1.82
C:N	< 25			28.14
GI (%)	> 90			139.21
Moisture (%)	< 60			37.15
Organic Matter (%)	Type AA > 50	Type A > 40	Type B > 30	53.26
pH	NA			8.11
Trace Elements	Type AA	Type A	Type B	R3
As	13		75	5.991
Co	34		150	0.655
Cr	210		1060	8.422
Cu	100		757	15.523
Mo	5		20	5.227
Ni	62		180	182.507
Se	2		14	0.158
Zn	500		1850	75.423
Cd	3		20	0.601
Hg	0.8		5	0.039
Pb	150		500	2.649

Table 3.7 Characterizations for Ratio 4 (R4)

	Standards			R4
Organic carbon	NA			43.29
Nitrogen	NA			2.17
C:N	< 25			19.95
GI (%)	> 90			146.88
Moisture (%)	< 60			35.57
Organic Matter (%)	Type AA > 50	Type A > 40	Type B > 30	54.72
pH	NA			8.15
Trace Elements	Type AA	Type A	Type B	R4
As	13		75	8.615
Co	34		150	< LD*
Cr	210		1060	6.970
Cu	100		757	21.204
Mo	5		20	1.929
Ni	62		180	49.253
Se	2		14	2.004
Zn	500		1850	103.475
Cd	3		20	0.691
Hg	0.8		5	< LD*
Pb	150		500	4.718

Table 3.8 Characterizations for Ratio 5 (R5)

	Standards			R5
Organic carbon	NA			46.91
Nitrogen	NA			1.10
C:N	< 25			42.65
GI (%)	> 90			152.06
Moisture (%)	< 60			34.84
Organic Matter (%)	Type AA > 50	Type A > 40	Type B > 30	60.45
pH	NA			8.12
Trace Elements	Type AA	Type A	Type B	R5
As	13		75	3.456
Co	34		150	0.454
Cr	210		1060	7.371
Cu	100		757	7.616
Mo	5		20	6.723
Ni	62		180	237.831
Se	2		14	< LD*
Zn	500		1850	56.768
Cd	3		20	1.059
Hg	0.8		5	< LD*
Pb	150		500	2.649

Table 3.9 Characterizations for Ratio 6 (R6)

	Standards			R6
Organic carbon	NA			49.02
Nitrogen	NA			1.39
C:N	< 25			35.27
GI (%)	> 90			143.05
Moisture (%)	< 60			52.05
Organic Matter (%)	Type AA > 50	Type A > 40	Type B > 30	43.64
pH	NA			8.09
Trace Elements	Type AA	Type A	Type B	R6
As	13		75	3.664
Co	34		150	0.649
Cr	210		1060	8.653
Cu	100		757	8.465
Mo	5		20	6.859
Ni	62		180	302.525
Se	2		14	< LD*
Zn	500		1850	47.466
Cd	3		20	0.229
Hg	0.8		5	< LD*
Pb	150		500	2.117

Table 3.10 Characterizations for Ratio 7 (R7)

	Standards			R7
Organic carbon	NA			39.30
Nitrogen	NA			3.49
C:N	< 25			11.26
GI (%)	> 90			160.24
Moisture (%)	< 60			15.8
Organic Matter (%)	Type AA > 50	Type A > 40	Type B > 30	44.02
pH	NA			8.01
Trace Elements	Type AA	Type A	Type B	R7
As	13		75	7.400
Co	34		150	0.759
Cr	210		1060	4.582
Cu	100		757	13.171
Mo	5		20	1.249
Ni	62		180	52.662
Se	2		14	1.033
Zn	500		1850	86.038
Cd	3		20	0.930
Hg	0.8		5	< LD*
Pb	150		500	3.985

Table 3.11 Characterizations for Ratio 8 (R8)

	Standards			R8
Organic carbon	NA			45.32
Nitrogen	NA			2.89
C:N	< 25			15.68
GI (%)	> 90			150.44
Moisture (%)	< 60			21.34
Organic Matter (%)	Type AA > 50	Type A > 40	Type B > 30	45.78
pH	NA			8.23
Trace Elements	Type AA	Type A	Type B	R8
As	13		75	4.805
Co	34		150	0.387
Cr	210		1060	2.864
Cu	100		757	6.750
Mo	5		20	1.484
Ni	62		180	57.849
Se	2		14	< LD*
Zn	500		1850	63.033
Cd	3		20	0.502
Hg	0.8		5	< LD*
Pb	150		500	2.776

In summary, R1, R2, and R4 qualify as Type B level compost products; R3, R5, and R6 do not qualify for any type of compost product; and R7 and R8 qualify for Type A level compost products. In order to further improve the quality of R7 and R8 to Type AA level, algal biodegradation was conducted.

3.3 Determination of Biodegradation Period

During the algal biodegradation process, all six parameters were tested every six days until some indexes reached a stabilized stage. Algal treatment was conducted for both R7 and R8. For each ratio, three different doses—5 ml, 10 ml, and 20 ml—of algal solution were added to 20 g mixtures respectively. All doses started at the same time with the same reaction conditions. The amount of algal solution added to the mixture was the only variable. During the 24-day biodegradation treatment, all six runs showed similar trends. Run 1 is discussed in detail as an example. Characterizations are shown in Tables 3.12 and 3.13 and Figures 3.2 to 3.5.

Table 3.12 Characterizations for Run 1 in a 24-Day Biodegradation Process

Days	C:N	Organic Carbon (%)	Nitrogen (%)	GI (%)	OM (%)	Moisture (%)	pH	Trace Elements
Standards	< 25	NA	NA	> 90	Type AA> 50; Type A> 40; Type B> 30	< 60	NA	See Table 3.13
D1	25.33	39.26	1.55	155.78	44.61	34.66	8.01	
D6	24.56	38.44	1.57	157.44	47.60	28.47	8.12	
D12	19.82	37.47	1.89	158.20	48.96	27.41	8.06	
D18	16.39	37.21	2.27	158.71	49.03	25.21	8.04	
D24	15.21	36.13	2.31	160.74	49.06	24.87	7.99	

Table 3.13 Trace Element Variations for Run 1 in a 24-Day Biodegradation Process

Trace Elements	Type AA	Type A	Type B	D1	D6	D12	D18	D24
As	13		75	7.318	7.319	7.403	7.289	7.311
Co	34		150	0.760	0.755	0.760	0.756	0.761
Cr	210		1060	4.579	4.580	4.577	4.567	4.584
Cu	100		757	13.168	13.170	13.165	13.169	13.171
Mo	5		20	1.243	1.239	1.245	1.244	1.248
Ni	62		180	52.666	52.659	52.660	52.655	52.664
Se	2		14	1.024	1.034	1.029	1.031	1.033
Zn	500		1850	85.978	86.042	86.040	86.036	86.033
Cd	3		20	0.926	0.935	0.925	0.933	0.914
Hg	0.8		5	< LD*	< LD*	< LD*	< LD*	< LD*
Pb	150		500	3.978	4.001	3.998	3.980	3.988

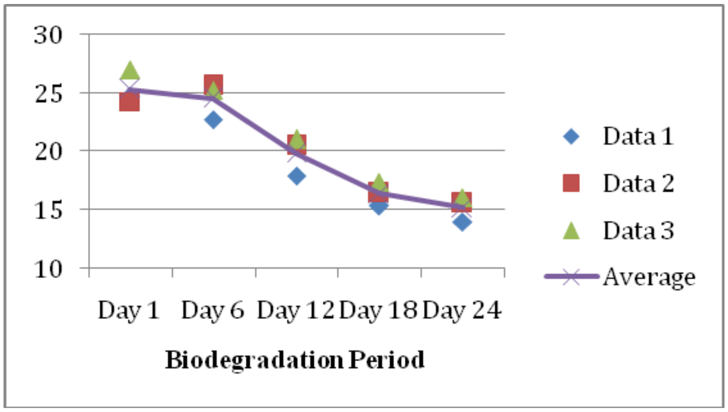

Figure 3.2 Run 1 C:N Variations in a 24-Day Algal Biodegradation Process

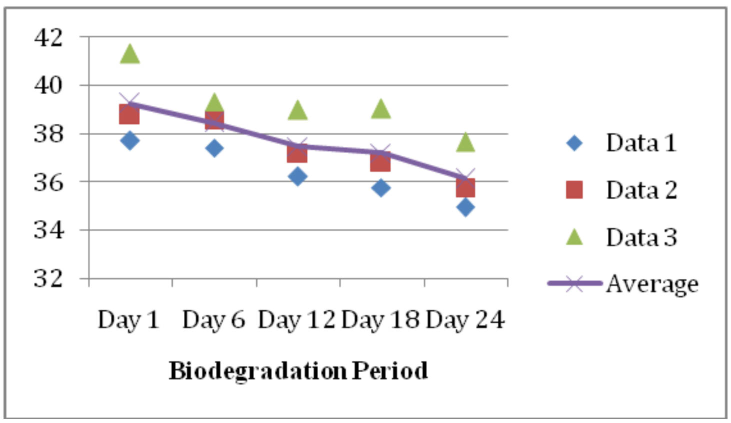

Figure 3.3 Run 1 Organic Carbon Variations in a 24-Day Algal Biodegradation Process

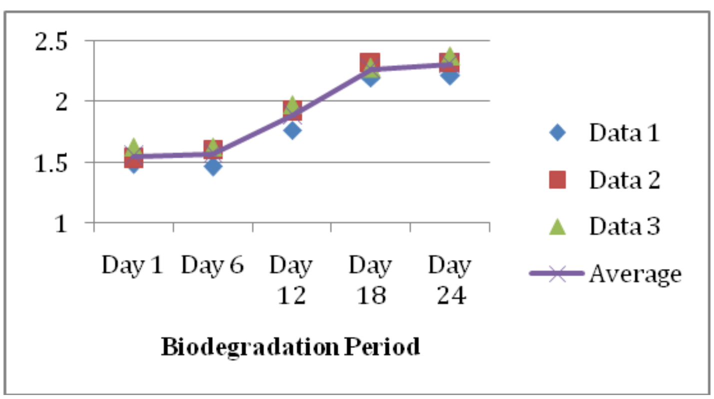

Figure 3.4 Run 1 Nitrogen Variations in a 24-Day Algal Biodegradation Process

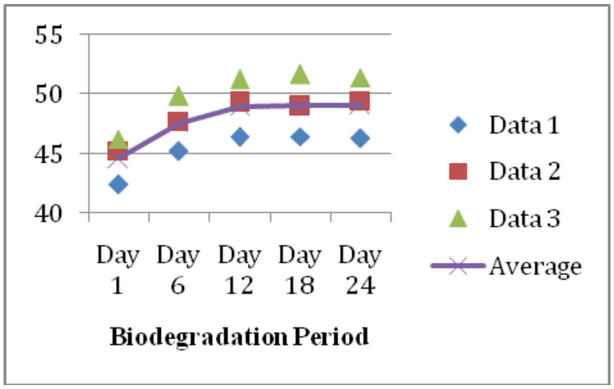

Figure 3.5 Run 1 OM Variations in a 24-Day Algal Biodegradation Process

After adding 5 ml algal solution to R7, many chemical qualities changed significantly. For C:N, there was no obvious difference in the first five days; but after six days, it decreased from 25.33 to 16.39, reaching a stable stage after 18 days. For nitrogen, there was also no significant difference in the first five days; however, it increased from 1.57% to 2.27% in the next 12 days, and then stabilized for the remaining days. For the OM, the curve increased dramatically in the first six days but was nearly flat after 12 days. There was no obvious increase in the next 12 days. The curve of organic carbon decreased from 39.26% to 36.13%. Overall, all the parameters reached a stable status after 18 days; this means that the algal biodegradation process was almost complete.

According to the standards established by CCME and BNQ, after 24 days of 5 ml algal biodegradation R7 can qualify as a Type A level compost product.The C:N for R7 decreased to 15.21, which is lower than 25; the nitrogen increased from 1.55% to 2.31%; the GI improved from 155.78 to 160.74; and the OM reached 49.06%.

In summary, for all six runs, the C:N and organic carbon did not show any obvious change in the first six days, but decreased gradually after that until a stable stage was reached; this happened in 18 days. For nitrogen, there was also no major difference in the first five days; however, it did increase considerably in the next 12 days, and remained stable. For the OM, the curve increased dramatically in the first six days but was close to being flat after 12 days, and showed no obvious increase in the next 12 days. Overall, the parameters reached a stable status after 18 days, which means that the algal biodegradation process was almost complete.

3.4 Determination of Algal Dose

From the previous discussion, it is clear that Anabaena played an important role in improving the qualities of the compost. However, it does not mean that the addition of more algae generates a better compost. As shown in Figures 3.6 to 3.9, the results of R7 (Runs 1-3) were discussed for four aspects: C:N, nitrogen, organic carbon, and organic matter.

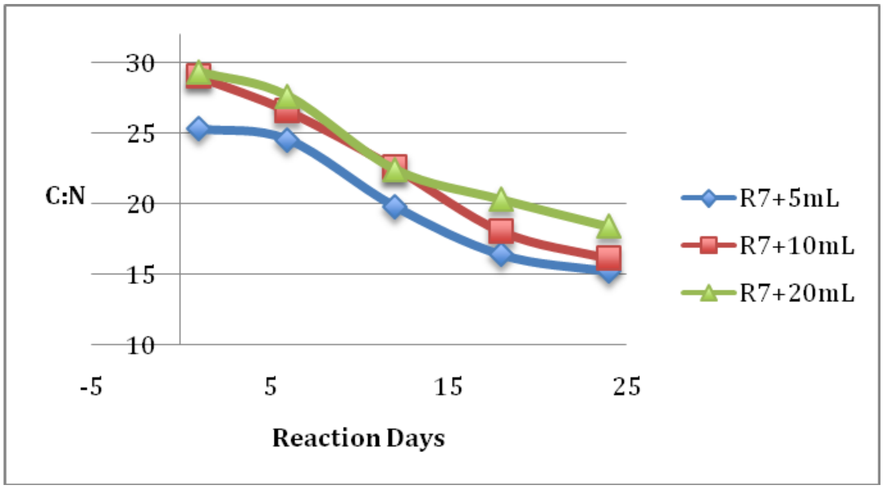

Figure 3.6 Comparison of C:N for Runs 1, 2, and 3

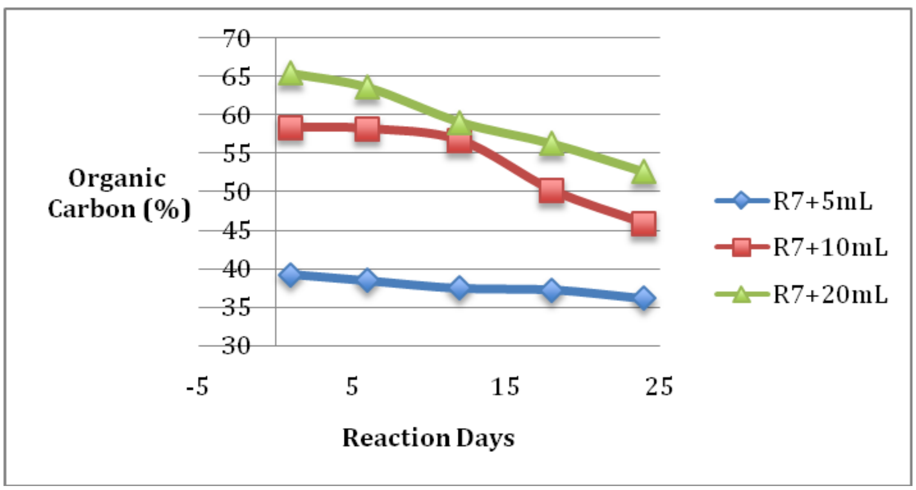

Figure 3.6 Comparison of Organic Carbon for Runs 1, 2, and 3

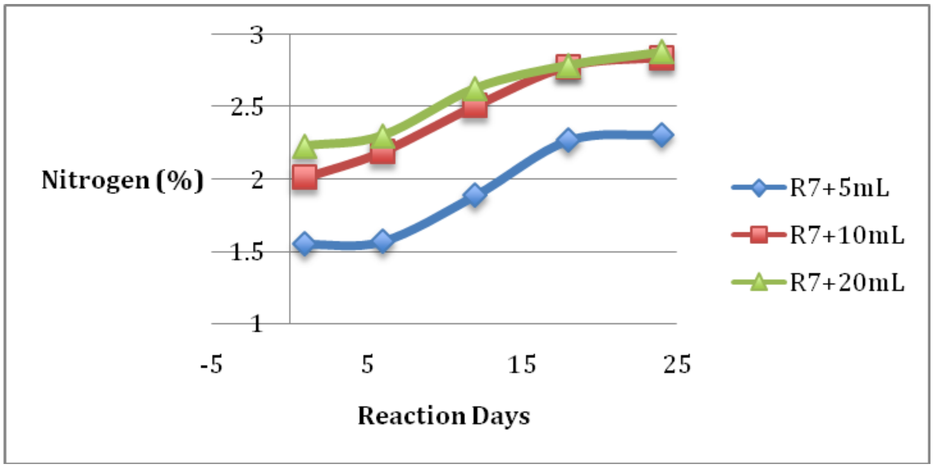

Figure 3.7 Comparison of Nitrogen for Runs 1, 2, and 3

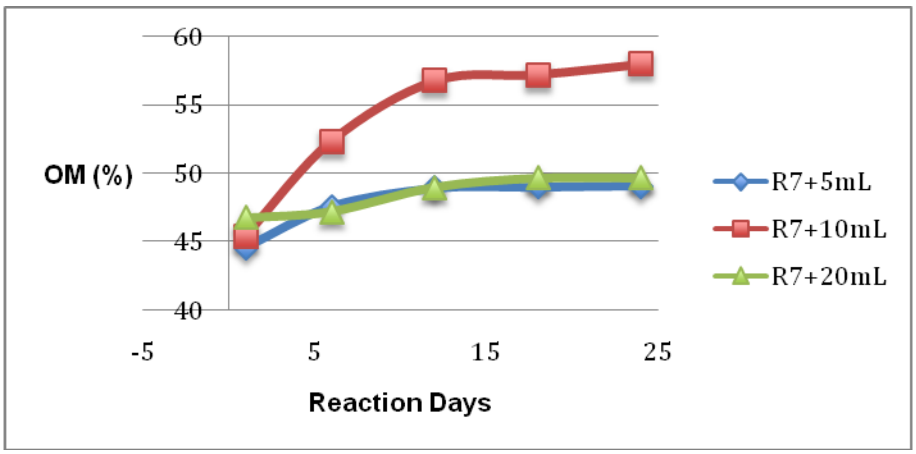

Figure 3.8 Comparison of Organic Matter for Runs 1, 2, and 3

Since there was too much water in a 20 ml algal solution, R7 does not qualify as a compost product; therefore, 20 ml was not regarded as a practical amount. Only 5 ml and 10 ml doses were considered as reasonable volumes. Between these two ratios, 10 ml is better than 5 ml. After a 24-day algal treatment, the nitrogen in Run 2 is much higher than in Run 1. For the OM, Run 1 is almost the same as Run 3; however, Run 2 had an obvious advantage. According to the standards, Run 1 was leveled as Type A but Run 2 as Type AA. The same results were obtained from R8 (Runs 4-6): Run 4 was leveled as Type A but Run 5 Type AA. Run 6 did not qualify as compost because of its high water content.

3.5 Classification of Composts

After mixing the raw materials of fly ash, sludge, and fish-waste compost in various ratios, three ratios met the standards for a Type B level compost product. Then, by adding different doses of algal solution two Type AA and two Type A level composts were generated after biodegradation. The classification of compost products is summarized in Table 3.14. Unfortunately, some mixtures still do not qualify as composts: R3, R5, R6, Runs 3 and 6.

Table 3.14 Classification of Compost Products

	Type AA	Type A	Type B
Compost products	Run 2 after 18 days biodegradation Run 5 after 18 days biodegradation	Run 1 after 18 days biodegradation Run 4 after 18 days biodegradation	R1 R2 R4

CHAPTER 4 CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

Each raw material—fish-waste compost, fly ash, and sludge—is not qualified to be compost directly by itself. Each has its own characteristics and the potential to generate a good-quality compost product after being mixed with each other in various ratios. According to the characterizations of the eight different mixing ratios, ratios 3, 5, and 6 did not qualify as compost products because of their over-the-limit concentrations for trace elements. Ratios 1, 2, and 4 did qualify, but only for a Type B level compost, based on the evaluation standards. Meanwhile, two Type A level compost products, ratios 7 and 8, were generated and did undergo algal biodegradation in order to obtain better chemical qualities. Three different amounts (5 ml, 10 ml, and 20 ml) of algal solution were added to both ratios 7 and 8 (each ratio is a 20-gram mixture) for algal biodegradation. Over a 24-day algae treatment process, a biodegradation curve was drawn based on the changes in the six parameters.

All the data and curves clarify that Anabaena has biodegradation ability and improves compost qualities. Anabaena can biodegrade most of the organic pollutants and stabilize more nitrogen into the compost. It can adjust the C:N to an ideal range. A 2:1 ratio of compost to algal solution has the best performance therefore both Runs 2 and 5 can be classified as Type AA level compost after treatment. Meanwhile, even though Runs 1 and 4 remain in Type A level compost, their OM content increases considerably. Both of these two ratios show similar biodegradation curves, which reflect an optimal reaction period of 18 days. After 18 days, biodegradation activities are almost completed and tend to be slow.

4.2 Significance of Research

This research provides an innovative method for the generation of different levels of compost products. Reaction conditions can be controlled in order to produce different types of compost based on application purpose. The contributions of this research include:

1) Compared with the conventional composting process, multiple resources were used: fly ash, sludge, and barren fish-waste compost after extraction;

2) Meanwhile, the reuse of these multiple resources offers another environmentally friendly way for waste management and recycling;

3) Algal biodegradation improved the chemical qualities, such as C:N and organic matter content, of the compost product;

4) This research shows how an environmental problem (algae bloom) can be used as a biotechnology (biodegradation) to generate a valuable agricultural product (compost); and

5) Multiple choices of compost products were available. Type B level or Type A level compost can be used for horticultural purposes, and Type AA level compost can be applied in agriculture.

Different demands from customers can be met by a diverse selection.

4.3 Recommendations for Future Work

Although this research brings a new scope to the study of compost, considerable work is needed to reveal a deeper understanding of biodegradation processes. Here are some recommendations for future research.

For the chemical qualities of the compost, this research mainly focused on the improvement of carbon to nitrogen ratio, organic matter, and nitrogen content. However, trace elements also affect the classification of compost. Due to difficulties in separating the algae from the compost, the concentration of trace elements in the compost did not decrease after biodegradation. It is recommended that more work be designed in order to reduce the concentration of trace elements in the compost samples.

In addition, the biodegradation process can be set up as a modeling program for a better control of reaction factors. Flexible adjustments of reaction conditions during the process might help with generating better compost products with less energy input and a shorter production period.

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